

NOVEL COMPONENTS FROM SECRETORY HAIRS
OF AZALEA LACE BUG *Stephanitis pyrioides*

(HEMIPTERA: TINGIDAE)

JAMES E. OLIVER, JOHN W. NEAL, JR., WILLIAM R. LUSBY,
JEFFREY R. ALDRICH, and JAN P. KOCHANISKY

Agricultural Research Service, U.S.D.A.
Beltsville, Maryland 20705

(Received November 9, 1984; accepted January 9, 1985)

Abstract—The azalea lace bug secretes a clear fluid from secretory setae on the antennae and globulated spines on the dorsal and lateral aspects of the abdomen. The secretion contains 2-alkyl-5-hydroxychromones, the corresponding chromanones and diketones, and straight-chain aldehydes and ketones.

Key Words—Heteroptera, Tingidae, *Stephanitis*, lace bug, setal exudate, chromones, chromanones.

INTRODUCTION

Nymphs and adults of the lace bug *Stephanitis pyrioides* (Scott) feed on the undersides of azalea leaves. In spite of the gregarious feeding and social habits of lace bugs (Drake and Ruhoff, 1965), neither parasites nor predators of nymphs of *S. pyrioides* have been reported. There are incidental reports of predation of *Stephanitis* species by an occasional hemipteran (e.g., Johnson, 1936), but Sheeley and Yonke (1977) failed to find any significant numbers of either parasites or predators for seven species of Missouri tingids. Although defensive substances associated with lace bugs have not been reported, it is known (Livingstone, 1978) that nymphs secrete a clear, slightly viscous fluid from setae or hairs that are generally distributed laterally and dorsally on abdominal protruberances as well as on segments of the antennae. These microdroplets are reported and remain in place on the end of the hairs (Figure 1). We here report the identification and synthesis of the major chemical constituents of secretions of nymphs of *S. pyrioides*.

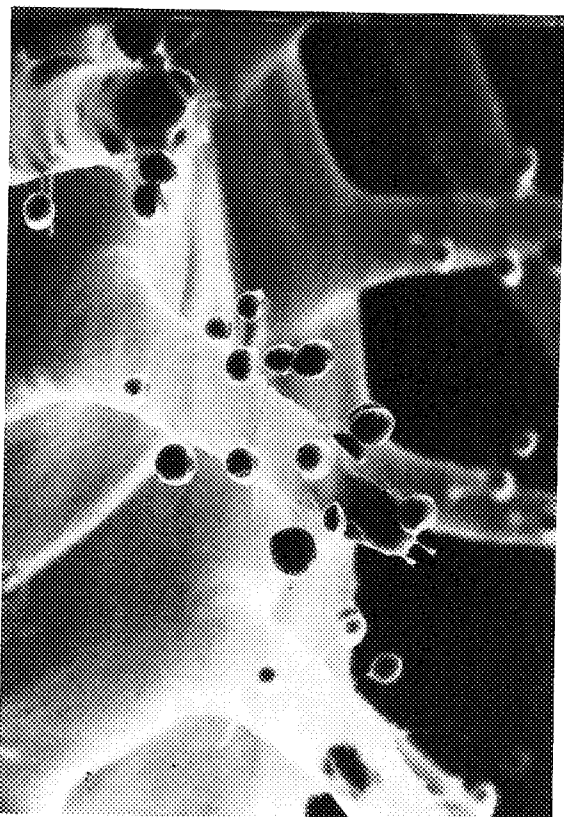


FIG. 1. Exuviae of *S. pyroides* fourth instar with tubercles (scoli) with globulated spines.

METHODS AND MATERIALS

Gas chromatography was performed on a Varian model 3700 instrument equipped with a flame ionization detector and a 13 m capillary DB-1 column (J & W Scientific, Inc.) with helium as carrier. The injector temperature was 270°C and the column was heated either isothermally or programmed over a range of 100–245°. Mass spectra were obtained from a Finnigan model 4510 GC-MS-DS fitted with a 30 mm × 0.32 mm ID DB-1 (0.25 μm film of methyl silicone) fused silica column. Spectra were collected at 70 eV and a source temperature of 150°. High-performance liquid chromatography was performed on a Spectra-Physics model 8700 system equipped with a 30 cm × 3.9 mm C₁₈ μ-Bondapak column eluted with 85% methanol containing 0.005 M tetrabutylammonium phosphate.

A colony of *Stephanitis pyroides* (Scott) was maintained in a greenhouse on container-grown azaleas (Krumme hybrid, "Blaauw's Pink"); third through fifth instar nymphs were used for collections. Microdroplets were blotted from the dorsal abdominal aspects of laboratory-reared nymphs with small strips of filter paper, and dichloromethane extracts of the filter paper were examined by capillary gas chromatography, gas chromatography-mass spectrometry, and high-performance liquid chromatography.

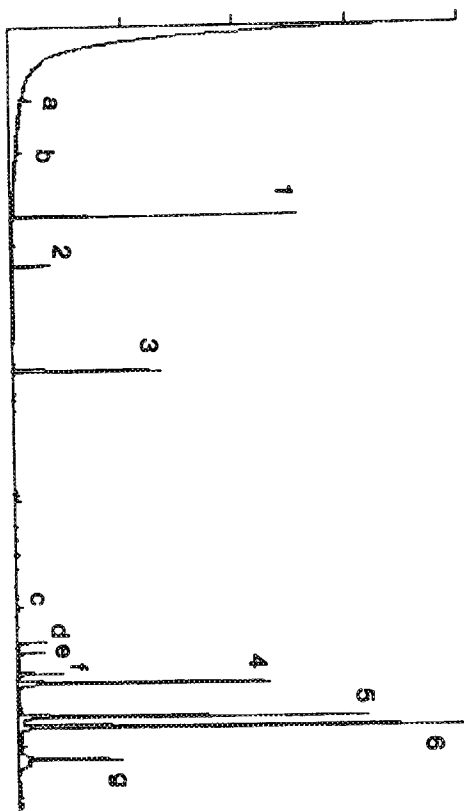
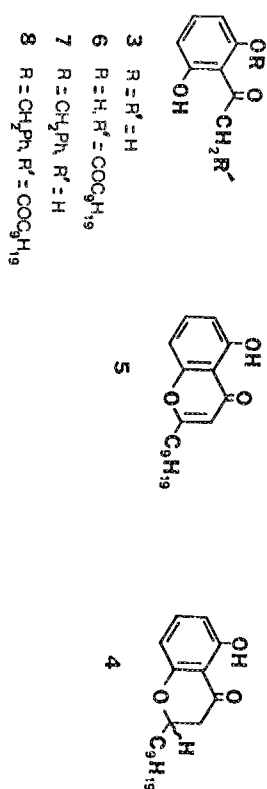


FIG. 2. Reconstructed ion chromatogram of compounds secreted by *S. pyroides*. The components assigned numbers were positively identified and are described in the text and in Scheme 1. Structures of the components assigned lowercase letters have not been confirmed but had been tentatively proposed to be: a, octanal; b, nonan-2-one; c, 2-heptyl-5-hydroxychroman-4-one; d, 2-heptyl-5-hydroxychromone; e, 2,6'-dihydroxy-2-octanoylacetophenone; f, unidentified; g, dioctyl phthalate.

RESULTS

A reconstructed ion chromatogram of the nymph exudate is shown in Figure 2. Decanal (1) and undecan-2-one (2) were initially identified by computer-selected matches of their mass spectra. The data system suggested 2',4'-dihydroxyacetophenone as a likely structure for the next component (m/z 152, 137). However, comparison with authentic (the 2',4'-, 2',5'-, and 2',6'-isomers were available from the Aldrich Chemical Company), showed that the secreted compound was in fact the 2',6'-isomer (3). The three later-eluting major components 4–6 were unknown, but their mass spectra indicated molecular weights of 290, 288, and 306, respectively (molecular weights of 4–6 were confirmed by chemical ionization mass spectrometry using both methane and ammonia as reagent gases); furthermore, an intense ion at m/z 137 (dihydroxybenzoyl¹⁷) in the electron impact mass spectrum of each suggested that all three were related to or derived from 3.

Both chromones (Eguchi, 1979; Ellis, 1977a) and chromanones (Van de Sande and Vandewalle, 1973; Ellis, 1977b) are known to undergo facile reverse Diels-Alder fragmentations upon electron impact; thus a 2-alkyl-5-hydroxy derivative of either ring system might be expected to produce a mass spectral



SCHEME 1. Structures and synthesis of identified compounds.

fragment of m/z 137 equivalent to that produced by 3. A reported (Tringali and Piattelli, 1982) mass spectrum of 5,7-dihydroxy-2-nonadecylchromone contains a series of homologous ions completely parallel to those found in the mass spectrum of 5, differing by 16 amu because of one less oxygen on the ring of 5 [principal ions of 5 occurred at m/z 288 (35%), 189 (100%), 176 (57%), 147 (10%), 137 (78%), 108 (12%)]. None of the alkyl ions produced by 5 suggested branching, and we proposed 5-hydroxy-2-nonylchromone as its structure (Scheme 1).

Compound 4 proved to be 2 amu heavier than 5. The electron impact mass spectrum of 4 contained a molecular ion at m/z 290 (44%) and fragments at 163 (93%), 137 (100%), 108 (12%), and we proposed the chromanone structure illustrated in Scheme 1.

The mass spectrum of the major component 6 had an ion (relative intensity 100%) at m/z 179, a moderately intense ion at m/z 137 (63%), and relatively weak ions at m/z 306 (molecular ion, 5%), 189 (9%), 152 (13%), and 108 (10%). Briefly warming a sample of the mixture with HCl resulted in conversion of 6 to 5, consistent with the known facile cyclization of such diketones to chromones (Ellis, 1977c).

Assignments of structures 1-3 were confirmed by comparing gas chromatographic retention times and mass spectra to those of commercial samples. Compounds 4-6 were synthesized as outlined in Scheme 1: monobenzyl ether 7 (Kametani and Kano, 1963) and ethyl decanoate were condensed with sodium hydride in pyridine (Cooke and Down, 1971) to give 8 (81%, mp 50.5-52.5°). Hydrogenolysis of 8 (Pd on carbon 1 atm in ethanol containing a little triethylamine) gave 6 as a very pale yellow solid, mp 71.5-73.5° after recrystallization from hexane plus a small amount of benzene [1H NMR ($CDCl_3$) δ 0.90 (t, 3H), 1.31 (m, 14H), 2.10 (m, 2H), 2.88 (s, 2H), 6.23-7.41 (m, 3H), 11.61 (s, 2H)]. A sample of 6 (0.28 g) was boiled 3-5 min in a mixture of conc. HCl (2 ml) and ethanol (4 ml); after cooling to 0°, the solid was collected and recrystallized

from wet methanol to give 0.21 g of 5, mp 51-52°. [1H]NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.29 (m, 14H), 2.55 (m, 2H), 6.09 (s, 1H), 6.63-7.63 (m, 3H), 12.45 (s, 1H).

As has been discussed by Ellis (1977d) and others, hydrogenation of the 2,3 double bond of chromones is slow, and competitive reduction of the carbonyl often occurs. To obtain chromanone 4, hydrogenation of 5 (Pd/C, EtOAc-EtOH, 1 atm) was followed by periodic gas chromatographic analyses until the relative concentration of 4 no longer increased. Silica gel chromatography (hexane with increasing increments of benzene) then gave a pure sample of *dl*-4 (eluted with 60-80% benzene) that was an oil room temperature but formed a white solid at 0°. [1H]NMR ($CDCl_3$) δ 0.89 (t, 3H), 0 (m, 16H), 2.69 (d, $J = 7$ Hz, 2H), 4.3 (m, 1H), 6.3-7.4 (m, 3H), 11.65 (1H). Gas chromatographic retention times and mass spectra of synthetic 4-6 perfectly matched those of the insect-derived materials. In addition, 5 and 6 were further confirmed by RP-HPLC; 4 was not detected in this particular sample.

A somewhat less laborious and more quantitative method of collecting the material was to simply dip the nymphs and/or their cast moult skins in a vial containing dichloromethane. However, such solutions were usually contaminated with varying amounts of additional components including saturated hydrocarbons that may have been of insect cuticle origin. From 25 cast skins from fifth-instar nymphs, we extracted an estimated (by GLC) total of 18 μ g 5 + 6, i.e., ca. 0.8 μ g/nymph. The average live weight of fifth-instar nymphs was determined to be 0.38 mg; thus 5 and 6 seem to constitute approximately 0.2% of their body weight.

Compounds 1, 4, 5, and 6 were readily discernible by GLC in nearly all samples examined, whereas the concentrations of 2 and 3 seemed somewhat more variable. Some cyclization of 6 to 5 was found to occur during GLC analyses; this was not a problem during HPLC analyses, however, and the latter demonstrated that both compounds were present in all insect-derived samples examined. The minor components indicated by lowercase letters in Figure 2 were lost in the baseline noise of many of the samples, and these compounds have not been investigated in detail. Their mass spectra are strongly suggestive of a homologous series of compounds with the alkyl groups shorter by two carbons.

Compounds 4-6 have not previously appeared in the literature. We considered the possibility that the insects were obtaining them from the azalea leaves, but we have no evidence that this was the case. Extraction of freeze-dried azalea leaves followed by column chromatography and GLC analysis of fractions with TLC R_f s corresponding to those of 4-6 indicated that measurable amounts of 4-6 were not present in the host plant. However, we have not ruled out the possibility that they might have been present as conjugates. In one case, we examined the secretion of a group of nymphs of *S. pyraoides* found locally on an ornamental rhododendron (*Rhododendron carawbiense* "Boursault," i.e., a

different species of host plant). The composition of this secretion was indistinguishable from that derived from the nymphs feeding on azaleas.

We believe that this constitutes the first identification of tingid exocrine compounds and the first report of either chromones or chromanones from insects. Studies on the function of these compounds are in progress as are investigations of secretions of additional species of lace bugs. Our preliminary results suggest that the rhododendron lace bug, *Stephanitis rhododendri* (Horvath), produces a mixture that contains diketone 6 and several related compounds. In contrast, those representatives of the *Corythucha* genus thus far examined [*C. cydoiae* Fitch (Hawthorn lace bug), *C. arcuata* Say (Oak lace bug), and *C. ulmi* Osborne and Drake (Elm lace bug)] have been found to produce a series of as yet unidentified compounds related to each other but apparently unrelated to those found in the *Stephanitis* genus.

REFERENCES

- COOKE, R. G., and DOWN, J. G. 1971. Colouring matters of Australian plants. XVI. Minor constituents of *Dianella revoluta* and *Stypantra grandis*. *Aust. J. Chem.* 24:1257-1265.
- DRAKE, C. J. and RUHOFF, F. A. 1965. Lace bugs of the world: A catalog (Hemiptera: Tingidae). *Smithson. Inst. USNM Bull.* 243:634 pp. (000).
- EGUCHI, S. 1979. Hydrogen transfer in the retro Diels-Alder fragmentation of oxygen-containing heterocyclic compounds. II. Chromones. *Org. Mass Spectrom.* 14:345-349.
- ELLIS, G. P. 1977. Chromenes, chromanones, and chromones. *Heterocycl. Compd.* 31:589.
- ELLIS, G. P. 1977b. *Chem. Heterocycl. Compd.* 31:222.
- ELLIS, G. P. 1977c. *Chem. Heterocycl. Compd.* 31:495-556.
- ELLIS, G. P. 1977d. *Chem. Heterocycl. Compd.* 31:256.
- JOHNSON, C. G. 1936. The biology of *Leptobrysa rhododendri* Horvath (Hemiptera: Tingidae), the rhododendron lace bug. *Ann. Appl. Biol.* 23:342-368.
- KAMEJANI, T., and KANO, S. 1963. Synthesis of heterocyclic compounds. LXXX. Synthesis of papaverine derivatives. 10. Synthesis of 1-(2,3-dihydroxyphenyl)- and 1-(2,6-dihydroxyphenyl)-3-methyl-6,7-methylenedioxyisoquinoline and its related compounds. *Yakugaku Zasshi* 83:356-360; *Chem. Abstr.* 59:7572b, 1963.
- LIVINGSTONE, D. 1978. On the body outgrowths and the phenomenon of "sweating" in the nymphal instars of Tingidae (Hemiptera: Heteroptera). *J. Nat. Hist.* 12:377-394.
- SHEELY, R. D., and YONKE, T. R. 1977. Biological notes on seven species of Missouri tingids (Hemiptera: Tingidae). *J. Kans. Entomol. Soc.* 50:342-356.
- TRINGALI, C., and PIATTELLI, M. 1982. Further metabolites from the brown alga *Zonari tournefortii*. *Gazz. Chim. Ital.* 112:465-468.
- VAN DE SANDE, C., and VANDEWALLE, M. 1973. Studies in organic mass spectrometry. XIV. Investigation of electron impact induced isomerisation of 2'-hydroxy-acetophenones and chroman-4-ones. *Bull. Soc. Chim. Belg.* 82:775-783.

IDENTIFICATION OF FEEDING STIMULANTS FOR BOLL WEEVILS FROM COTTON BUDS AND ANTHERS¹

G. H. MCKIBBEN,² M. J. THOMPSON,³ W. L. PARKOTT,²
A. C. THOMPSON,² and W. R. LUSBY³

²Boll Weevil Research Lab.

Agricultural Research Service USDA

P.O. Box 5367, Mississippi State, Mississippi 39762

³Insect Physiology Lab.

Agricultural Research Service USDA

Beltsville, Maryland 20705

(Received August 17, 1984; accepted January 9, 1985)

Abstract—Column chromatography of the pentane extract of freeze-dried cotton buds or anthers yielded a wax-sterol ester fraction that exhibited potent feeding stimulant activity for the cotton boll weevil. The waxes of the wax-sterol ester mixture were responsible for the feeding activity. Saponification of the wax-sterol ester fraction yielded about 15% alcohols and 85% sterols. A C_{18:1} alcohol, dihydrophytol, phytol, and geranylgeraniol constituted 15, 36, 26, and 23%, respectively, of the total alcohols, implicating certain of their long-chain esters as feeding stimulants. Several esters of dihydrophytol, phytol, and geranylgeraniol were identified among the waxes by GC-MS. Certain phytol, geranylgeraniol, and oleyl alcohol esters containing C₁₂ to C₂₆ acid moieties were synthesized and were found to induce high feeding stimulant activity in the cotton boll weevil.

Key Words—Boll weevil, *Anthonomus grandis*, Coleoptera, Curculionidae, feeding stimulants, cotton buds, anthers, phytol, geranylgeraniol esters, phytol oleate, phytol dodecanoate.

INTRODUCTION

Since Keller et al. (1962) reported the presence of feeding stimulant(s) for boll weevils, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae), in water extracts of cotton squares, considerable work has been done toward the identi-

¹In cooperation with the Mississippi Agricultural and Forestry Experiment Station.